THE IDENTIFICATION AND CHARACTERIZATION OF BACTERIOPHAGES WITH THE ELECTRON MICROSCOPE

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Bacteriophages, or bacterial viruses, are a group of viruses reproducing in the presence of living bacterial cells. Bacteriophages are particulate, and convincing evidence exists that (1) one particle of phage is sufficient to originate the lysis of a bacterial cell; in the lysis, a variable number of new phage particles (an average of 100 or more) are liberated per cell;[1] (2) the elementary particles of each phage strain seem to have a characteristic particle size as determined by any one of various indirect methods of investigation (ultrafiltration,[2] radiosensitivity,[3] diffusion,[4]) and diameters ranging from 10 to 100 mμ have been obtained for the various strains de-

pending on the method of investigation, although diffusion experiments occasionally yield still smaller values.

The electron microscope has recently been applied with success to the study of viruses[5] and it therefore seemed desirable to attempt such a study of bacterial viruses, particularly since they offer favorable possibilities for the identification of the virus particles through a study of the reaction between the individual particles and the bacterial cell under the microscope. Indeed, a number of short reports have been published recently by German authors[6, 7] in which round particles have been described as corresponding to the phage particles, although Ruska[7] shows pictures of "sperm-shaped" particles from a phage suspension adhering to a bacterial membrane. From this evidence alone he is unable to decide whether these are particles of phage or bacterial products.

We have undertaken an investigation of the problems of phage structure, size, reproduction and lytic activity by means of the RCA electron microscope. Research on the last items is still in progress. The present report concerns itself with the identification and the morphological analysis of a number of strains of phage particles and their adsorption on sensitive bacterial cells. The results are illustrated by some of the electron micrographs (Plates I and II) which have brought to light many extremely interesting features. Details of the material and methods used will soon be published.

I. Bacteriophage anti-coli. PC (particle diameter by diffusion 44 mγ, Kalmanson and Bronfenbrenner[8]; by x-irradiation 50 mμ, Luria and Exner, unpublished).

Micrographs of high titer suspensions, figures 1, 2, 4, 5 and 6, show the constant presence of particles of extremely constant and characteristic aspect. They consist of a round "head," and a much thinner "tail," which gives them a peculiar sperm-like appearance. The "head" is not homogeneous but shows an internal structure consisting of a pattern of granules, distinguished by their higher electron scattering power. Deviations from the usual symmetrical internal pattern may be due to varying orientation of the particles or to other factors as yet unknown. The diameter of the head appears to be about 80 mμ; the tail is about 130 mμ long.

EXPLANATION OF PLATE

PLATE I

1. Electron micrograph of particles from a high titer suspension of bacteriophage anti-coli PC. × 38,000.
2. Particles from a high titer suspension of bacteriophage anti-coli PC. × 84,000.
3. *Escherichia coli* from suspension in distilled water. × 17,000.
4. *Escherichia coli* in suspension of bacteriophage anti-coli PC for ten minutes. × 17,500.

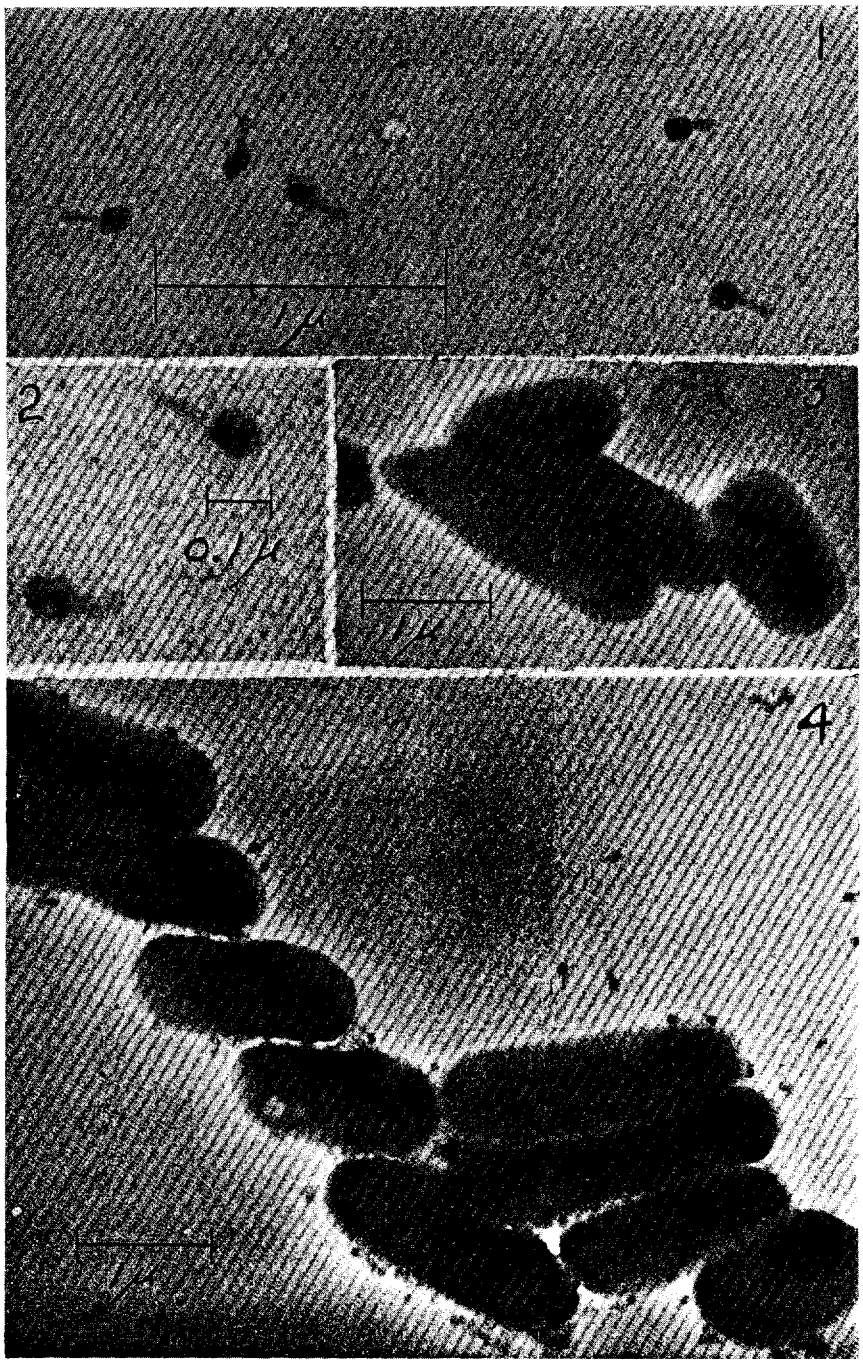

PLATE I

This gives a size which is in fair agreement with the figures deduced from the radiosensitivity method. On the other hand, it is possible that the size as determined by x-rays corresponds more closely to the size of the granules.

When allowed to stand a few minutes in the presence of sensitive bacterial cells *Escherichia coli*, strain PC (Fig. 3), the particles described above are readily adsorbed (Figs. 4 and 5). They appear to stick to the bacteria either by the head or by the tail. Other conditions remaining constant, the number of particles adsorbed on a bacterium increases with the time of contact, although it is difficult at the present time to differentiate between adsorption and reproduction of the particles on the cell wall. By allowing the phage to stay in contact with bacteria for a time of the order of the minimum time of lysis (21 minutes for PC phage, Delbrück and Luria[1]) it is possible to observe bacterial cells extensively damaged, surrounded by a very large number of particles, probably newly formed (Fig. 6).

II. Bacteriophage anti-coli P 28, also active on *Escherichia coli* strain PC (particle size: irradiation, 36 mμ, Luria and Exner.[3]

Round particles are visible in the suspensions of this phage which are somewhat smaller than those described for phage PC (about 50 mμ in diameter). An extremely thin tail, although difficult to demonstrate with certainty in the reproductions, seems to be visible in many instances. In many micrographs the head is almost completely filled by a dense internal structure. These particles, too, are readily adsorbed on sensitive bacterial cells.

III. Bacteriophage anti-staphylococcus 3K (particle size: by ultrafiltration and ultracentrifugation 50–75 mμ, Elford;[2] by irradiation 48 mμ, Luria and Exner.[3]

Owing to technical reasons, the conditions for successful micrographing are here less favorable. Nevertheless, the presence of approximately round particles of proper size has been established in preparations of this page also.

We are inclined to identify the particles described above with the actual particles of bacteriophage for the following reasons: (*a*) They are always present in highly active phage suspensions and missing in any control suspensions (media, bacterial cultures, bacterial filtrates, etc.); (*b*) they are readily adsorbed by the bacterial cells of the susceptible strain and fail to be adsorbed by other bacteria; (*c*) the size from a given strain is uniform and corresponds essentially to measurements by indirect methods; (*d*) the structure of both the "head" and the "tail" is characteristic of the strain of phage; (*e*) preliminary experiments on the lysis process seem to demonstrate the liberation of these particles from the lysing bacteria.

Conclusions.—We do not want to discuss here the bearing of the above described results on the problem of the nature of bacteriophage and of viruses in general. We limit ourselves to pointing out the extreme interest of the finding of such constant and relatively elaborate structural differen-

tiation in objects of supposedly macromolecular nature. This result is of equal interest in the field of genetics, since genes, together with viruses, are currently supposed to be macromolecular entities.

The correspondence of the particle size as directly portrayed in the electron microscope with the results of indirect methods is also very remarkable. although it does not exclude the possibility of phage activity being sometimes associated with smaller particles. It is worth while emphasizing that the results of the present investigation, together with the recently published results of irradiation of bacteriophages, represent most desirable evidence for the validity of the so-called "hit theory" for the determination of the "sensitive volume" in sub-light-microscopic biological objects. This conclusion, too, seems to be interesting from the point of view of genetics, since the "hit theory," although widely criticized, has been used for calculating the approximate value of the dimensions of genes.

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EXPLANATION OF PLATE

PLATE II

5. *Escherichia coli* in suspension of bacteriophage anti-coli PC for 20 minutes. × 14,500.

6. *Escherichia coli* in suspension of bacteriophage anti-coli PC for 20 minutes. × 12,500.

7 and 8. Particles from a high titer suspension of bacteriophage anti-coli P28. × 38,000.

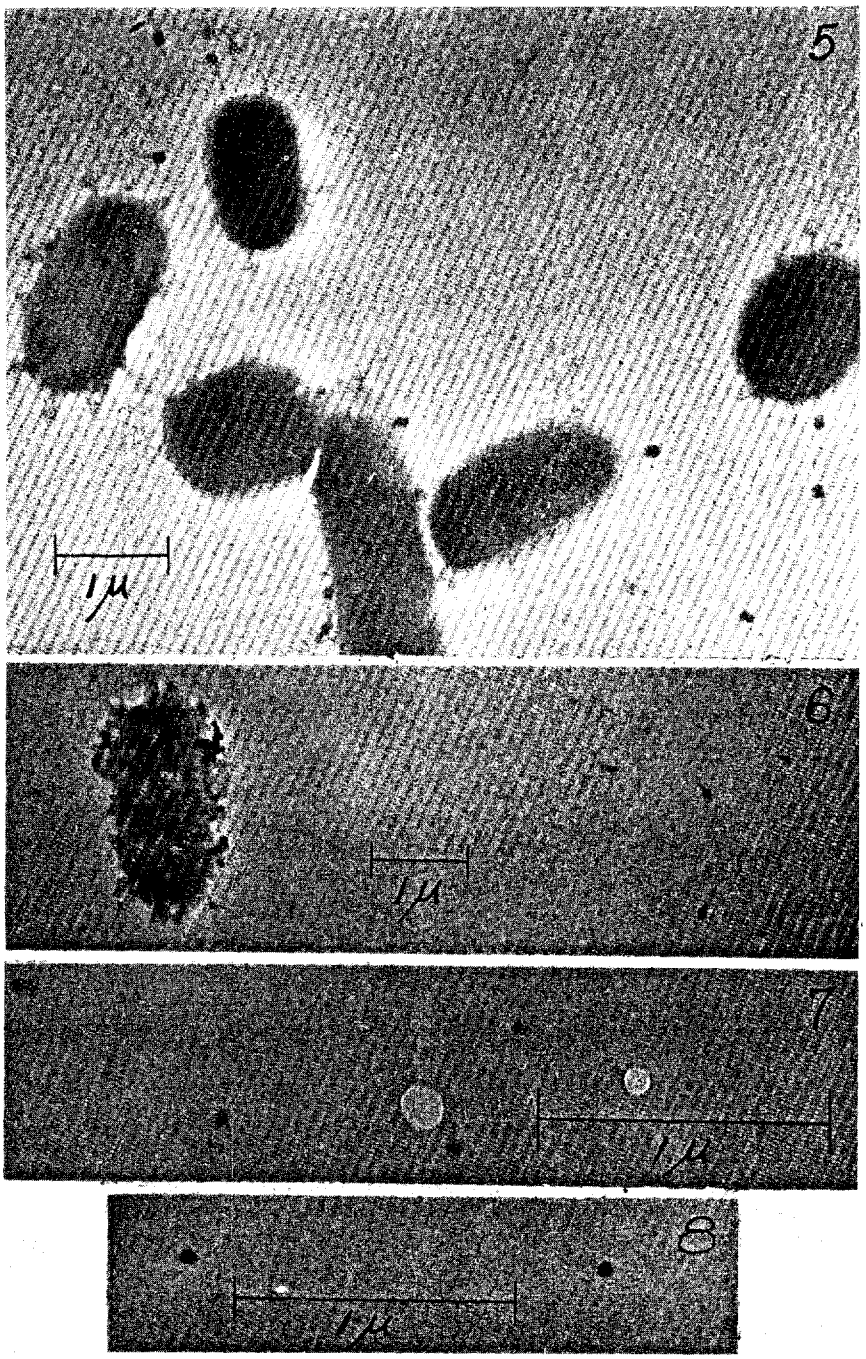

PLATE II